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Single amino-acid changes in HIV envelope affect viral tropism and receptor binding.

Cordonnier A, Montagnier L, Emerman M.

Unite d'Oncologie Virale (CNRS UA 1157), Institut Pasteur, Paris, France.

Infection by the human immunodeficiency virus (HIV) is initiated by the binding of its extracellular envelope glycoprotein, gp120, to the CD4 antigen on target cells. To map the residues of the HIV-1 glycoprotein that are critical for binding and to analyse the effects of binding on viral infectivity, we created 15 mutations in a region of gp120 that is important for binding to CD4 (refs 4,5). We find that substitution of a single amino acid (tryptophan at position 432) can abrogate CD4 binding and that virus carrying this mutation is non-infectious. By contrast, other amino-acid changes in the same region do not affect CD4 binding but restrict viral tropism: virions containing isoleucine substitutions at position 425 lose their ability to infect a monocyte cell line (U937 cells) but can still infect T-lymphocyte cell lines (CEM, SUP-T1) and activated human peripheral blood lymphocytes. These results indicate that cellular tropism of HIV can be influenced by a single amino-acid change in gp120.

PMID: 2475780 [PubMed - indexed for MEDLINE]

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Induction of cross-reactive antibodies against a self protein by immunization with a modified self protein containing a foreign T helper epitope.

Dalum I, Jensen MR, Gregorius K, Thomasen CM, Elsner HI, Mouritsen S.

M&E Biotech A/S, Horsholm, Denmark.

Self proteins are handled in the same way as foreign proteins by antigen presenting cells, but because of T-cell tolerance the presentation of self peptides does not normally lead to T cell activation. By providing physically linked T-cell help it is possible to overcome the B cell non-responsiveness toward self antigens. We have shown previously that a very potent antibody response, cross-reactive with a self protein, can be rapidly induced by immunizing with a recombinant immunogen consisting of the self protein with a foreign immunodominant T helper epitope inserted into its sequence (Dalum, I., Jensen, M. R., Hindersson, P., Elsner, H. I. and Mouritsen, S. (1996) J. Immnunol. 157, 4796). In this study we compare this approach for inducing autoantibodies against a self protein with the traditional method of conjugating the self antigen to a foreign carrier protein. The highly conserved self protein ubiquitin with an inserted epitope from ovalbumin (UbiOVA) is used as a model protein and compared to two traditionally conjugated immunogens consisting of ubiquitin chemically conjugated to a peptidic T helper epitope or to ovalbumin. The traditionally conjugated immunogens induce much slower and low titered ubiquitin specific antibody responses than the recombinant construct which also is capable of inducing antibodies directed against a much broader range of potential ubiquitin B cell determinants than the chemically conjugated immunogens. All three constructs are processed by antigen presenting cells and ovalbumin derived T cell epitopes are presented to T helper cells. From these observations it seems likely that the presence of non-shielded autologous B cell determinants on the immunogen is critical for the ability to induce a strong autoantibody response with a diverse fine specificity. Furthermore, the ubiquitin specific antibodies induced by UbiOVA contain higher levels of IgG2a/b relative to IgG1 compared to the conjugates. We therefore speculate that the insertion of a T cell epitope directly into the self antigen could possibly induce an immune response with a different Th1/Th2 balance than a response induced with traditional conjugates.

PMID: 9566759 [PubMed - indexed for MEDLINE]

CD4-dependent, antibody-sensitive interactions between HIV-1 and its co-receptor CCR-5.

Trkola A, Dragic T, Arthos J, Binley JM, Olson WC, Allaway GP, Cheng-Mayer C, Robinson J, Maddon PJ, Moore JP.

The Aaron Diamond AIDS Research Centre, The Rockefeller University, New York 10016, USA.

The beta-chemokine receptor CCR-5 is an essential co-factor for fusion of HIV-1 strains of the non-syncytium-inducing (NSI) phenotype with CD4+ T-cells. The primary binding site for human immunodeficiency virus (HIV)-1 is the CD4 molecule, and the interaction is mediated by the viral surface glycoprotein gp120 (refs 6, 7). The mechanism of CCR-5 function during HIV-1 entry has not been defined, but we have shown previously that its beta-chemokine ligands prevent HIV-1 from fusing with the cell. We therefore investigated whether CCR-5 acts as a second binding site for HIV-1 simultaneously with or subsequent to the interaction between gp120 and CD4. We used a competition assay based on gp120 inhibition of the binding of the CCR-5 ligand, macrophage inflammatory protein (MIP)-1beta, to its receptor on activated CD4+ T cells or CCR-5-positive CD4- cells. We conclude that CD4 binding, although not absolutely necessary for the gp120-CCR-5 interaction, greatly increases its efficiency. Neutralizing monoclonal antibodies against several sites on gp120, including the V3 loop and CD4-induced epitopes, inhibited the interaction of gp120 with CCR-5, without affecting gp120-CD4 binding. Interference with HIV-1 binding to one or both of its receptors (CD4 and CCR-5) may be an important mechanism of virus neutralization.

PMID: 8906796 [PubMed - indexed for MEDLINE]

Biological and immunological properties of human immunodeficiency virus type 1 envelope glycoprotein: analysis of proteins with truncations and deletions expressed by recombinant vaccinia viruses.

Earl PL, Koenig S, Moss B.

Laboratories of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.

The effects of C-terminal and internal deletions on the synthesis, transport, biological properties, and antigenicity of the human immunodeficiency virus type 1 envelope protein were determined. A family of recombinant vaccinia viruses that express N-terminal overlapping env proteins of 204, 287, 393, 502 (full-length gp120), 635, 747, and 851 (full-length gp160) amino acids was constructed. All of the proteins were detected in intra- and extracellular forms which differed in the extent of glycosylation. The 747- and 851-amino-acid proteins were cleaved, were expressed on the surface of infected cells, and bound CD4. The 635-amino-acid env protein was cleaved inefficiently, and both the precursor and product were secreted, indicating absence of the transmembrane sequence. The 635- as well as the 502-amino-acid protein, which was also largely secreted, could still bind CD4. Unexpectedly, the 393-amino-acid protein was anchored in the plasma membrane, but neither it nor smaller proteins bound to soluble CD4. When amino acids at the gp120-gp41 junction were deleted, proteolytic cleavage of gp160 did not occur. Nevertheless, gp160 was inserted into the plasma membrane and bound soluble CD4. The predominant conserved B-cell epitopes were mapped to gp41 and the C terminus of gp120, whereas cytotoxic T-cell epitopes were distributed throughout the length of the glycoproteins.

PMID: 1985202 [PubMed - indexed for MEDLINE]

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Neutralization of primary human immunodeficiency virus type 1 isolates by the broadly reactive anti-V3 monoclonal antibody, 447-52D.

Conley AJ, Gorny MK, Kessler JA 2nd, Boots LJ, Ossorio-Castro M, Koenig S, Lineberger DW, Emini EA, Williams C, Zolla-Pazner S.

Department of Antiviral Research, Merck Research Laboratories, West Point, Pennsylvania 19486.

Human monoclonal antibody 447-52D binds to the V3 determinant of the human immunodeficiency virus type 1 (HIV-1) gp120 external glycoprotein. Its binding requires the expression of the GPxR sequence at the center of the V3 domain. HIV-1 variants that are adapted to replication in T-lymphoid cell lines and express this sequence motif are efficiently neutralized by the antibody (M. K. Gorny, A. J. Conley, S. Karwowska, A. Buchbinder, J.-Y. Xu, E. A. Emini, S. Koenig, and S. Zolla-Pazner, J. Virol. 66:7538-7542, 1992). In the present study, the antiviral activity of 447-52D was further defined with regard to its ability to mediate neutralization of primary HIV-1 clinical isolates. Again, the antibody was found to potently neutralize those isolates that expressed the binding sequence. We confirmed that this determinant is commonly expressed by virus isolates belonging to the subtype (clade) B sequence classification. As such, 447-52D may be useful for prophylactic and immunotherapeutic intervention. In addition, the study demonstrated that neutralization of primary HIV-1 isolates is possible if mediated by an appropriate antibody.

PMID: 7933081 [PubMed - indexed for MEDLINE]

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Neutralization of diverse human immunodeficiency virus type 1 variants by an anti-V3 human monoclonal antibody.

Gorny MK, Conley AJ, Karwowska S, Buchbinder A, Xu JY, Emini EA, Koenig S, Zolla-Pazner S.

Department of Pathology, New York University Medical School, New York 10016.

The third variable region (V3) of the HIV-1 gp120 envelope glycoprotein is thought to induce potent neutralizing antibodies which are generally defined as type specific and reactive with individual viral isolates. In contrast, the CD4-binding domain is thought to induce neutralizing antibodies that are group specific and capable of neutralizing all isolates of HIV-1. However, in this study, we used a panel of human monoclonal antibodies to these regions of gp120 which displays specificities and neutralizing activities that challenge these tenets. In particular, we used a human monoclonal antibody to the V3 domain with exceptionally potent and broad neutralizing activity against many diverse HIV-1 isolates. The anti-CD4-binding domain antibodies, on the other hand, showed a more restricted pattern of activity.

PMID: 1433529 [PubMed - indexed for MEDLINE]

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An IgG human monoclonal antibody that reacts with HIV-1/GP120, inhibits virus binding to cells, and neutralizes infection.

Posner MR, Hideshima T, Cannon T, Mukherjee M, Mayer KH, Byrn RA.

Department of Medicine, Roger Williams Medical Center, Providence, RI 02908.

A human mAb (HmAb) termed F105 was obtained by fusion of antibody-producing EBV-transformed cells with the HMMA2.11TG/O cell line. F105 is an IgG1 kappa antibody that binds to the surfaces of cells infected with all HIV-1 strains tested: MN, RF, IIIB, and SF2, but not uninfected cells. The HmAb immunoprecipitates GP120 from all four strains. F105 does not react with denatured GP120 on Western blots, but does react with viral lysates and purified GP120 dotted onto nitrocellulose filter paper under nondenaturing conditions. rGP120 from SF2 and soluble rCD4 inhibit antibody binding to infected cells in a dose-dependent manner. F105 inhibits the binding of free, infectious virions to uninfected HT-H9 cells with 50% of maximal (100%) inhibition at approximately 1 microgram/ml. F105 inhibits infection of HT-H9 cells by 100 tissue culture infective dose 50% units of MN and IIIB strains with 50% inhibition at concentrations of HmAb readily achievable in man. It appears that the F105 HmAb reacts with a conformationally defined epitope on HIV-1/GP120 that is exposed on the free virion and is important for binding to the cell surface by the virion. The epitope, which is immunogenic in humans, appears to be within, or topographically near, the CD4-binding site. F105 and the F105 epitope are potentially useful in therapy and in the design of peptide or anti-Id based vaccines; monitoring of the expression of the Id may prove useful in evaluating immune responses in infected individuals or vaccinated volunteers.

PMID: 1710248 [PubMed - indexed for MEDLINE]

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Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160.

Berman PW, Gregory TJ, Riddle L, Nakamura GR, Champe MA, Porter JP, Wurm FM, Hershberg RD, Cobb EK, Eichberg JW.

Department of Immunobiology, Genentech, Inc., South San Francisco, California 94080.

The development of a vaccine to provide protective immunity to human immunodeficiency virus type 1 (HIV-1), the virus causing AIDS, would be the most practical method to control its spread. Subunit vaccines consisting of virus envelope glycoproteins, produced by recombinant DNA technology, are effective in preventing viral infections. We have now used this approach in the development of a candidate AIDS vaccine. Chimpanzees were immunized with recombinant forms of the HIV-1 glycoproteins gp120 and gp160 produced in Chinese hamster ovary cells, and then challenged with HIV-1. The control and the two animals immunized with the gp160 variant became infected within 7 weeks of challenge. The two animals immunized with the gp120 variant have shown no signs of infection after more than 6 months. These studies demonstrate that recombinant gp120, formulated in an adjuvant approved for human use, can elicit protective immunity against a homologous strain of HIV-1.

PMID: 2190095 [PubMed - indexed for MEDLINE]

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Characterization of a discontinuous human immunodeficiency virus type 1 gp120 epitope recognized by a broadly reactive neutralizing human monoclonal antibody.

Thali M, Olshevsky U, Furman C, Gabuzda D, Posner M, Sodroski J.

Department of Pathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115.

While one hypervariable, linear neutralizing determinant on the human immunodeficiency virus type 1 (HIV-1) gp120 envelope glycoprotein has been well characterized, little is known about the conserved, discontinuous gp120 epitopes recognized by neutralizing antibodies in infected individuals. Here, the epitope recognized by a broadly reactive neutralizing monoclonal antibody (F105) derived from an HIV-1-infected patient was characterized by examining the effects of changes in conserved gp120 amino acids on antibody reactivity. The F105 epitope was disrupted by changes in gp120 amino acids 256 and 257, 368 to 370, 421, and 470 to 484, which is consistent with the discontinuous nature of the epitope. Three of these regions are proximal to those previously shown to be important for CD4 binding, which is consistent with the ability of the F105 antibody to block gp120-CD4 interaction. Since F105 recognition was more sensitive to amino acid changes in each of the four identified gp120 regions than was envelope glycoprotein function, replication-competent mutant viruses that escaped neutralization by the F105 antibody were identified. These studies identify a conserved, functional HIV-1 gp120 epitope that is immunogenic in man and may serve as a target for therapeutic or prophylactic intervention.

PMID: 1717717 [PubMed - indexed for MEDLINE]

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Effects of mutations in hyperconserved regions of the extracellular glycoprotein of human immunodeficiency virus type 1 on receptor binding.

Cordonnier A, Riviere Y, Montagnier L, Emerman M.

Unite d'Oncologie Virale (Centre National de la Recherche Scientifique, UA1157), Institut Pasteur, Paris, France.

Sequence comparison of the human immunodeficiency virus type 1 and type 2 env genes revealed the presence of six linear regions in the extracellular glycoprotein that are highly conserved. To investigate the functional significance of these regions, we made short deletions in each and assayed the ability of the mutated proteins to bind CD4 antigen. Small deletions in four of the highly conserved regions drastically reduced receptor binding. Some deletions interfered with the maturation of the envelope glycoprotein, but maturation did not necessarily correlate with the ability to bind CD4 antigen.

PMID: 2550679 [PubMed - indexed for MEDLINE]

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Conformational epitope on gp120 important in CD4 binding and human immunodeficiency virus type 1 neutralization identified by a human monoclonal antibody.

Ho DD, McKeating JA, Li XL, Moudgil T, Daar ES, Sun NC, Robinson JE.

Aaron Diamond AIDS Research Center, New York University School of Medicine, New York 10016.

A human monoclonal antibody designated 15e is reactive with the envelope glycoprotein (gp120) of multiple isolates of human immunodeficiency virus type 1 (HIV-1). Antibody 15e also neutralizes HIV-1 with broad specificity and blocks gp120 binding to CD4. Characterization of the 15e epitope shows that it is conformation dependent and is distinct from previously recognized functional domains of gp120, suggesting that this epitope represents a novel site important for HIV-1 neutralization and CD4 binding. These findings have implications for the development of a vaccine for AIDS.

PMID: 1702163 [PubMed - indexed for MEDLINE]

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CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5.

Wu L, Gerard NP, Wyatt R, Choe H, Parolin C, Ruffing N, Borsetti A, Cardoso AA, Desjardin E, Newman W, Gerard C, Sodroski J.

LeukoSite, Inc., Cambridge, Massachusetts 02142, USA.

For efficient entry into target cells, primary macrophage-tropic and laboratory-adapted human immunodeficiency viruses type 1 (HIV-1) require particular chemokine receptors, CCR-5 and CXCR-4, respectively, as well as the primary receptor CD4 (refs 1-6). Here we show that a complex of gp120, the exterior envelope glycoprotein, of macrophage-tropic primary HIV-1 and soluble CD4 interacts specifically with CCR-5 and inhibits the binding of the natural CCR-5 ligands, macrophage inflammatory protein (MIP)-1alpha and MIP-1beta (refs 7, 8). The apparent affinity of the interaction between gp120 and CCR-5 was dramatically lower in the absence of soluble CD4. Additionally, in the absence of gp120, an interaction between a two-domain CD4 fragment and CCR-5 was observed. A gp120 fragment retaining the CD4-binding site and overlapping epitopes was able to interact with CCR-5 only if the V3 loop, which can specify HIV-1 tropism and chemokine receptor choice, was also present on the molecule. Neutralizing antibodies directed against either CD4-induced or V3 epitopes on gp120 blocked the interaction of gp120-CD4 complexes with CCR-5. These results suggest that HIV-1 attachment to CD4 creates a high-affinity binding site for CCR-5, leading to membrane fusion and virus entry.

PMID: 8906795 [PubMed - indexed for MEDLINE]

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Relationship of the human immunodeficiency virus type 1 gp120 third variable loop to a component of the CD4 binding site in the fourth conserved region.

Wyatt R, Thali M, Tilley S, Pinter A, Posner M, Ho D, Robinson J, Sodroski J.

Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, Massachusetts.

Neutralizing antibodies that recognize the human immunodeficiency virus gp120 exterior envelope glycoprotein and are directed against either the third variable (V3) loop or conserved, discontinuous epitopes overlapping the CD4 binding region have been described. Here we report several observations that suggest a structural relationship between the V3 loop and amino acids in the fourth conserved (C4) gp120 region that constitute part of the CD4 binding site and the conserved neutralization epitopes. Treatment of the gp120 glycoprotein with ionic detergents resulted in a V3 loop-dependent masking of both linear C4 epitopes and discontinuous neutralization epitopes overlapping the CD4 binding site. Increased recognition of the native gp120 glycoprotein by an anti-V3 loop monoclonal antibody, 9284, resulted from from single amino acid changes either in the base of the V3 loop or in the gp120 C4 region. These amino acid changes also resulted in increased exposure of conserved epitopes overlapping the CD4 binding region. The replication-competent subset of these mutants exhibited increased sensitivity to neutralization by antibody 9284 and anti-CD4 binding site antibodies. The implied relationship of the V3 loop, which mediates post-receptor binding steps in virus entry, and components of the CD4 binding region may be important for the interaction of these functional gp120 domains and for the observed cooperativity of neutralizing antibodies directed against these regions.

PMID: 1279195 [PubMed - indexed for MEDLINE]

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Lack of correlation between soluble CD4-induced shedding of the human immunodeficiency virus type 1 exterior envelope glycoprotein and subsequent membrane fusion events.

Thali M, Furman C, Helseth E, Repke H, Sodroski J.

Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, Massachusetts.

The noncovalent association of the gp120 and gp41 envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1) is disrupted by soluble CD4 binding, resulting in shedding of the gp120 exterior envelope glycoprotein. This observation has led to the speculation that interaction of gp120 with the CD4 receptor triggers shedding of the exterior envelope glycoprotein, allowing exposure of gp41 domains necessary for membrane fusion steps involved in virus entry or syncytium formation. To test this hypothesis, a set of HIV-1 envelope glycoprotein mutants were used to examine the relationship of soluble CD4-induced shedding of the gp120 glycoprotein to envelope glycoprotein function in syncytium formation and virus entry. All mutants with a threefold or greater reduction in CD4-binding ability exhibited marked decreases in gp120 shedding in response to soluble CD4, even though several of these mutants exhibited significant levels of envelope glycoprotein function. Conversely, most fusion-defective mutants with wild-type gp120-CD4 binding affinity, including those with changes in the V3 loop, efficiently shed gp120 following soluble CD4 binding. Thus, soluble CD4-induced shedding of gp120 is not a generally useful marker for conformational changes in the HIV-1 envelope glycoproteins necessary for the virus entry or syncytium formation processes. Some gp120 mutants, despite being expressed on the cell surface and capable of efficiently binding soluble CD4, exhibited decreased gp120 shedding. These mutants were still sensitive to neutralization by soluble CD4, indicating that, for envelope glycoproteins exhibiting high affinity for soluble CD4, competitive inhibition may be more important than gp120 shedding for the antiviral effect.

PMID: 1501286 [PubMed - indexed for MEDLINE]

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Amino acid residues of the human immunodeficiency virus type I gp120 critical for the binding of rat and human neutralizing antibodies that block the gp120-sCD4 interaction.

McKeating JA, Thali M, Furman C, Karwowska S, Gorny MK, Cordell J, Zolla-Pazner S, Sodroski J, Weiss RA.

Institute of Cancer Research, Chester Beatty Laboratories, London, U.K.

We have characterized the discontinuous epitopes recognized by two rat and three human neutralizing monoclonal antibodies (mAb) by examining the effect of single amino acid changes in conserved residues of gp120 on mAb recognition. A human mAb derived from an infected individual, 448D, and two rat mAbs, 39.13g and 39.3b, respectively, derived by immunization with native recombinant gp120, recognize similar epitopes. Recognition of the envelope glycoproteins by these mAbs was affected by changes in gp120 amino acid residues 88, 113, 117, 257, 368, or 370. The gp120 amino acids 257, 368, and 370 have previously been reported to be important for CD4 binding, which is consistent with the ability of these mAbs to block the gp120-CD4 interaction. Residues 88, 113, and 117 are not thought to be important for CD4 binding, suggesting that the antibody epitopes overlap, but are distinct from, the CD4 binding region. We also found that some alterations in gp120 residues 88, 117, 368, or 421 reduced the ability of polyclonal sera from HIV-1-infected individuals to inhibit the interaction of the mutant gp120 glycoproteins with soluble CD4. Thus, changes in the HIV-1 gp120 glycoprotein that minimally affect the receptor binding may allow escape from neutralizing antibodies directed against the CD4 binding region.

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Discontinuous, conserved neutralization epitopes overlapping the CD4-binding region of human immunodeficiency virus type 1 gp120 envelope glycoprotein.

Thali M, Furman C, Ho DD, Robinson J, Tilley S, Pinter A, Sodroski J.

Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, Massachusetts.

Monoclonal antibodies have been isolated from human immunodeficiency virus type 1 (HIV-1)-infected patients that recognize discontinuous epitopes on the gp120 envelope glycoprotein, that block gp120 interaction with the CD4 receptor, and that neutralize a variety of HIV-1 isolates. Using a panel of HIV-1 gp120 mutants, we identified amino acids important for precipitation of the gp120 glycoprotein by three different monoclonal antibodies with these properties. These amino acids are located within seven discontinuous, conserved regions of the gp120 glycoprotein, four of which overlap those regions previously shown to be important for CD4 recognition. The pattern of sensitivity to amino acid change in these seven regions differed for each antibody and also differed from that of the CD4 glycoprotein. These results indicate that the CD4 receptor and this group of broadly neutralizing antibodies recognize distinct but overlapping gp120 determinants.

PMID: 1380099 [PubMed - indexed for MEDLINE]

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Contribution of disulfide bonds in the carboxyl terminus of the human immunodeficiency virus type I gp120 glycoprotein to CD4 binding.

Lekutis C, Olshevsky U, Furman C, Thali M, Sodroski J.

Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115.

The carboxyl half of the HIV-1 gp120 glycoprotein, which has been implicated in binding to the CD4 receptor, contains two disulfide bonds linking cysteine residues 378-445 and 385-418. To examine the necessity of these disulfide bonds for the formation and/or maintenance of a gp120 glycoprotein competent for CD4 binding, we created mutants of a soluble form of gp120 in which combinations of these cysteine residues were altered. The mutant glycoproteins were examined for export from the expressing cell and for CD4 binding ability. Mutant gp120 molecules lacking both disulfide bonds were not stably expressed or exported. However, mutants for which either disulfide bond could form were exported and were fully competent for CD4 binding. In some cases, the presence of one of the pair of linked cysteines exerted more detrimental effects on export or CD4 binding than did alteration of both cysteines. Thus, the evaluation or the contribution of a particular disulfide bond to a phenotype should include studies in which both cysteines involved in the bond are simultaneously altered.

PMID: 1738091 [PubMed - indexed for MEDLINE]

Cooperativity of neutralizing antibodies directed against the V3 and CD4 binding regions of the human immunodeficiency virus gp120 envelope glycoprotein.

Thali M, Furman C, Wahren B, Posner M, Ho DD, Robinson J, Sodroski J.

Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115.

Human immunodeficiency virus type 1 (HIV-1) infection elicits neutralizing antibodies directed against two discrete regions of the gp120 exterior envelope glycoprotein: the third variable (V3) loop and the CD4 binding region. Monoclonal antibodies directed against these two regions demonstrated additive or, in some cases, weakly synergistic neutralization of HIV-1 infection. Cooperativity in virus neutralization was also observed for some gp120 mutants that, in the absence of anti-V3 loop antibodies, were relatively resistant to neutralization by antibodies directed against the CD4 binding region. Although the binding of some anti-V3 region monoclonal antibodies increased the recognition of the multimeric envelope glycoproteins by anti-CD4 binding antibodies, this enhanced binding was not predictive of the degree of cooperativity observed in virus neutralization. These results suggest that elicitation of both types of neutralizing antibodies should increase the efficacy of vaccine preparations.

PMID: 1588493 [PubMed - indexed for MEDLINE]

1: J Virol 1993 Aug;67(8):4785-96 Related Articles, Links

Immunochemical analysis of the gp120 surface glycoprotein of human immunodeficiency virus type 1: probing the structure of the C4 and V4 domains and the interaction of the C4 domain with the V3 loop.

Moore JP, Thali M, Jameson BA, Vignaux F, Lewis GK, Poon SW, Charles M, Fung MS, Sun B, Durda PJ, et al.

Aaron Diamond AIDS Research Center, New York University School of Medicine, New York 10016.

We have probed the structure of the C4 and V3 domains of human immunodeficiency virus type 1 gp120 by immunochemical techniques. Monoclonal antibodies (MAbs) recognizing an exposed gp120 sequence, (E/K)VGKAMYAPP, in C4 were differentially sensitive to denaturation of gp120, implying a conformational component to some of the epitopes. The MAbs recognizing conformation-sensitive C4 structures failed to bind to a gp120 mutant with an alteration in the sequence of the V3 loop, and their binding to gp120 was inhibited by both V3 and C4 MAbs. This implies an interaction between the V3 and C4 regions of gp120, which is supported by the observation that the binding of some MAbs to the V3 loop was often enhanced by amino acid changes in an around the C4 region.

PMID: 7687303 [PubMed - indexed for MEDLINE]

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Further characterization of an antigenic site of HIV-1 gp120 recognized by virus neutralizing human monoclonal antibodies.

Schutten M, McKnight A, Huisman RC, Thali M, McKeating JA, Sodroski J, Goudsmit J, Osterhaus AD.

Laboratory of Immunobiology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

OBJECTIVE: The aim of this study is to characterize antigenic sites on HIV-1 gp120 which may be important for the development of active and passive immunization strategies against HIV-1 infection. DESIGN: Two HIV-1-seropositive individuals were selected from the Amsterdam cohort and Epstein-Barr virus (EBV)-transformed B cells were generated from their peripheral blood mononuclear cells, which produce HIV-1-specific human monoclonal antibodies (HuMAb). METHODS: HuMAb were generated and selected based on their reactivities with native gp120. Reactivity with HIV-1 strains from phylogenetically different subfamilies was determined by immunostaining and virus neutralization assays. Specificity for the CD4-binding site was tested by an inhibition enzyme-linked immunosorbent assay and amino acids (aa) involved in the binding of the HuMAb were identified with a set of gp120 molecules with single aa substitutions. RESULTS: Three HuMAb (GP13, GP44, GP68) were generated, all recognizing a conserved conformation dependent epitope within, or topographically near, the CD4-binding site of gp120. HuMAb GP13 and GP68 neutralized a broad range of HIV-1 strains from phylogenetically different subfamilies, whereas HuMAb GP44 exhibited a more restricted pattern of neutralizing activity. The patterns of gp120 aa involved in their binding were unique for each of these HuMAb. CONCLUSIONS: The pattern of reactivities of these three HIV-1-neutralizing HuMAb developed in these studies is similar to, but distinct from other human and rodent MAb that recognize this antigenic site of HIV-1 gp120.

PMID: 7689324 [PubMed - indexed for MEDLINE]

1: J Virol 1993 Jul;67(7):3978-88 Related Articles, Links

Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120-CD4 binding.

Thali M, Moore JP, Furman C, Charles M, Ho DD, Robinson J, Sodroski J.

Department of Pathology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts.

Interaction with the CD4 receptor enhances the exposure on the human immunodeficiency type 1 gp120 exterior envelope glycoprotein of conserved, conformation-dependent epitopes recognized by the 17b and 48d neutralizing monoclonal antibodies. The 17b and 48d antibodies compete with anti-CD4 binding antibodies such as 15e or 21h, which recognize discontinuous gp120 sequences near the CD4 binding region. To characterize the 17b and 48d epitopes, a panel of human immunodeficiency virus type 1 gp120 mutants was tested for recognition by these antibodies in the absence or presence of soluble CD4. Single amino acid changes in five discontinuous, conserved, and generally hydrophobic regions of the gp120 glycoprotein resulted in decreased recognition and neutralization by the 17b and 48d antibodies. Some of these regions overlap those previously shown to be important for binding of the 15e and 21h antibodies or for CD4 binding. These results suggest that discontinuous, conserved epitopes proximal to the binding sites for both CD4 and anti-CD4 binding antibodies become better exposed upon CD4 binding and can serve as targets for neutralizing antibodies.

PMID: 7685405 [PubMed - indexed for MEDLINE]

Medline Abstract

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DD Ho, JA McKeating, XL Li, T Moudgil, ES Daar, NC Sun, and JE Robinson

Conformational epitope on gp120 important in CD4 binding and human immunodeficiency virus type 1 neutralization identified by a human monoclonal antibody.

J. Virol., January 1, 1991; 65(1): 489-93. [Abstract]

Aaron Diamond AIDS Research Center, New York University School of Medicine, New York 10016.

A human monoclonal antibody designated 15e is reactive with the envelope glycoprotein (gp120) of multiple isolates of human immunodeficiency virus type 1 (HIV-1). Antibody 15e also neutralizes HIV-1 with broad specificity and blocks gp120 binding to CD4. Characterization of the 15e epitope shows that it is conformation dependent and is distinct from previously recognized functional domains of gp120, suggesting that this epitope represents a novel site important for HIV-1 neutralization and CD4 binding. These findings have implications for the development of a vaccine for AIDS.

Publication Type:

- Journal article

MeSH Terms:

- Antibodies, Monoclonal
- Antigen-Antibody Complex
- Antigens, CD4
- Dithiothreitol
- Epitopes
- HIV Envelope Protein gp120
- HIV-1
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- Neutralization Tests
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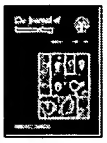

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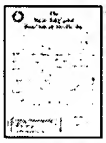

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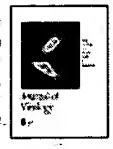

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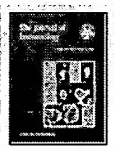

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Synergistic Neutralization of Simian-Human Immunodeficiency Virus SHIV-vpu^{+} by Triple and Quadruple Combinations of Human Monoclonal Antibodies and High-Titer Anti-Human Immunodeficiency Virus Type 1 Immunoglobulins

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Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein.

Cao J, Bergeron L, Helseth E, Thali M, Repke H, Sodroski J.

Division of Human Retrovirology, Dana-Farber Cancer Institute, Boston, Massachusetts.

Changes were introduced into conserved amino acids within the ectodomain of the human immunodeficiency virus type 1 (HIV-1) gp41 transmembrane envelope glycoprotein. The effect of these changes on the structure and function of the HIV-1 envelope glycoproteins was examined. The gp41 glycoprotein contains an amino-terminal fusion peptide (residues 512 to 527) and a disulfide loop near the middle of the extracellular domain (residues 598 to 604). Mutations affecting the hydrophobic sequences between these two regions resulted in two phenotypes. Some changes in amino acids 528 to 562 resulted in a loss of the noncovalent association between gp41 and the gp120 exterior glycoprotein. Amino acid changes in other parts of the gp41 glycoprotein (residues 608 and 628) also resulted in subunit dissociation. Some changes affecting amino acids 568 to 596 resulted in envelope glycoproteins partially or completely defective in mediating membrane fusion. Syncytium formation was more sensitive than virus entry to these changes. Changes in several amino acids from 647 to 675 resulted in higher-than-wild-type syncytium-forming ability. One of these amino acid changes affecting tryptophan 666 resulted in escape from neutralization by an anti-gp41 human monoclonal antibody, 2F5. These results contribute to an understanding of the functional regions of the HIV-1 gp41 ectodomain.

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Development of high potency universal DR-restricted helper epitopes by modification of high affinity DR-blocking peptides.

Alexander J, Sidney J, Southwood S, Ruppert J, Oseroff C, Maewal A, Snoke K, Serra HM, Kubo RT, Sette A, et al.

Cytel Corporation, San Diego, California 92121.

Pan DR-binding peptides engineered by introducing anchor residues for different DR motifs within a polyalanine backbone bound 10 of 10 DR molecules tested, with affinities, in most cases, in the nanomolar range. Because of the small methyl group exposed for T cell recognition, these peptides were poor immunogens but effective blockers of DR-restricted antigen presentation. Introduction of bulky and charged residues at positions accessible for T cell recognition yielded extremely powerful Pan DR epitope peptides (PADRE). These peptides elicited powerful responses in vitro from human peripheral blood mononuclear cells (PBMC). Because these cells also cross-react on certain mouse class II alleles, we could also demonstrate that PADRE peptides are active in vivo. In one example of their capacity to elicit T help, they were approximately 1000 times more powerful than natural T cell epitopes. We propose that PADRE peptides may be useful in the development of subunit vaccines.

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